Emergent Fluoroquinolone Resistance in Viridans Streptococcal Species in Cancer Patients

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Abstract

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Objectives: Viridans group streptococci (VGS) are known to be associated with morbidity and mortality in cancer and hematopoietic cell transplant recipients (HCT), and reports have described the development of fluoroquinolone (FQ) resistant VGS in these populations. To understand recent trends, we evaluated the frequency of VGS bacteremia and prevalence of FQ-resistance at a large tertiary medical system with a dedicated cancer center.

Methods: We identified patients from the University of Washington Medical Center (UW) and Seattle Cancer Care Alliance (SCCA) with any detected VGS from blood cultures between 1/2006 and 12/2012 for inclusion in the study. All patients were ≥18 years of age and categorized into cancer and non-cancer populations. Microbiologic data were abstracted from laboratory databases; demographic and outcome data on cancer patients were collected through electronic medical record review. Fisher's exact and Mann Whitney tests were used to compare study populations and multivariate logistic regression used to identify risk factors for FQ resistance in cancer patients with VGS.

Results: We identified 261 patients that had VGS identified from blood cultures from 2006-2012, of whom 165/261 (63%) were cancer patients. Among cancer patients, most had a primary diagnosis of a hematological malignancy (142/165 [86%]), and 69/165 (42%) were HCT recipients. We identified an increase in VGS cases among cancer patients over time. Of VGS identified at a species level (n=150), *S. mitis* was more common in the cancer population (72/95 [75.8%] vs. 29/55 [52.7%], p=0.004). Antimicrobial sensitivities for VGS indicated no statistical differences in resistance to penicillin (9.0% vs. 8.1%), clindamycin (11.3% vs. 7.1%), erythromycin (34.0% vs. 20.2%), ceftriaxone (1.9% vs. 6.1%) and vancomycin (0% in both) when comparing cancer to non-cancer patients. Overall, 102 of 261 isolates (39%) were FQ resistant. However, nearly all resistant isolates were from cancer patients (99/165 [60%] vs. 3/96 [3%]; $p \leq 0.001$). Calendar year, HCT, primary oncologic diagnosis, chemotherapeutic regimen, and levofloxacin prophylaxis were associated in univariate analyses, but the only significant risk factor in multivariate analyses was prophylactic levofloxacin use (OR 397 [95% CI 43.6-3628]). When excluding levofloxacin from the model, only calendar years 2009-2012 (OR 4.2 to 7.1) was associated with resistant VGS. Overall mortality was higher in patients with FQ resistant VGS [41.7% vs. 27.9%; p=0.018], but associated and attributable mortality were not significantly different [12% vs. 6.7%; p=0.36]) when compared to those without resistance.

<u>Conclusion</u>: In a large tertiary healthcare system, we demonstrated that an increase in VGS bacteremia cases was almost exclusively found in cancer and HCT patients. Not surprisingly, the use of levofloxacin for neutropenic prophylaxis at the cancer center was associated with FQ resistance.

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I. Background and Significance:

Cancer therapeutics, including hematopoietic cell transplant, result in immune deficits which, in turn, contribute to increased incidence and severity of infections.(1-5) The use of neutropenic prophylaxis with antibiotics reduces rates of gram negative (GNR) bacteremia, episodes of fever during chemotherapy, and most importantly, infection-related and all-cause mortality.(6, 7) Current national and international infection prevention guidelines recommend the use of fluoroquinolones as initial agents for neutropenic prophylaxis.(8-10)

Viridans group streptococci species (VGS), normally considered commensal bacteria of the gastrointestinal tract (11-13), are associated with bacteremia, sepsis, multi-organ failure and death in patients receiving chemotherapy or HCT.(3, 7, 14-17) Established risk factors for this infectious complication include neutropenia, mucositis and use of gastric acid suppression.(7, 9, 17-20) Incidence rates of VGS bacteremia vary between cancer centers ranging from 5-30%.(3, 7, 14-17, 21-28)

Emerging evidence suggests a link between fluoroquinolone use and VGS in cancer patients in some (7, 14, 27, 29-31), but not all centers.(1, 4, 5, 23, 32, 33) Reports of fluoroquinolone-resistant VGS in patients on fluoroquinolone prophylaxis are also concerning when considering the widespread use of such strategies.(27, 30, 34) Interestingly, although these data suggest potential negative outcomes of fluoroquinolone prophylaxis, there are few studies that have evaluated VGS and emergence of resistance following major shifts in neutropenic prophylactic regimens.

To better understand long term trends in VGS at a center with routine fluoroquinolone-based neutropenic prophylactic strategy in cancer and transplant patients, we conducted a longitudinal study of patients treated at the Seattle Cancer Care Alliance (SCCA) in Seattle, Washington between 2006 and 2012. To evaluate the role of fluoroquinolone prophylaxis on VGS resistance, we compared the frequency of VGS bacteremia and fluoroquinolone resistance in cancer patients compared to other patients at the general University of Washington (UW) hospital. Finally, we evaluated fluoroquinolone resistance and assessed potential risk factors for resistance within cancer patients with documented VGS.

II. Methods

Study population

Patients with VGS documented from blood cultures processed at the UW Microbiology laboratory between 1/2006 and 12/2012 were included in the study. All patients with VGS were categorized into cancer patients and non-cancer patients. Patients were considered non-cancer patients if they did not have oncological disease at the time of the VGS event but underwent care at UW Medical Center (UWMC) or affiliated clinics. Patients were considered cancer patients if they were currently diagnosed with an oncologic disease at the time of the VGS event. Cancer patients were categorized further into hematopoietic cell transplant (HCT) patients and hematology/oncologic patients (Hem/Onc). Patients $\leq$ 18 years of age at the time of infection were excluded from analyses.

Microbiologic assessment and Antibacterial prophylaxis

All patients have blood cultures drawn by UW/SCCA clinical services at the discretion of the treating healthcare provider. At the SCCA, it is recommended that two sets of blood cultures are taken during initial evaluation of any fever, and that daily blood cultures continue unless fevers subside or alternative sources for fever have been identified. Frequency of repeat blood cultures in patients with bacteremia is driven by clinical appearance and documentation of negative follow-up blood cultures. In addition, since glucocorticoids blunt febrile responses, HCT recipients who are treated with glucocorticoids ($\geq$ 0.5mg/kg) for graft-versus-host disease (GVHD) undergo bi-weekly blood cultures as part of center-based standard practice (surveillance blood cultures); following tapering of steroids to $\leq$ 0.5mg/kg, these are discontinued. The majority of cancer patients had cultures drawn through available access sites, central catheters or ports, and peripheral blood cultures are less frequent; non-cancer patients had blood cultures most frequently from peripheral sites.

Adult HCT recipients and Hem/Onc patients receive levofloxacin prophylaxis during periods of neutropenia unless intolerant or allergic, in which case they receive ceftazidime. During documented neutropenic fever, the SCCA guidelines recommend ceftazidime as the first-line antibiotic therapy, and vancomycin is often co-administered in the event of moderate or severe mucositis. However, decisions regarding antibiotic choice depend upon the patient's clinical presentation and are made by the primary team.

Many cancer patients and all HCT patients also receive trimethoprim-sulfamethoxazole (TMP), dapsone or atovaquone for PCP prophylaxis and acyclovir or valacyclovir for herpes simplex/varicella zoster virus prophylaxis. All HCT patients and the majority of hematologic malignancy patients receive antifungal prophylaxis with fluconazole, or an extended spectrum azole (voriconazole or posaconazole). HCT recipients undergo CMV preemptive surveillance as has been previously described.(35)

Data collection

All microbiologic data on both cancer and non-cancer VGS positive patients were abstracted from UWMC Microbiology Laboratory and SCCA databases. Demographic and clinical outcome data were collected for all cancer patients through electronic medical record (EMR) chart review; demographic data were not collected as part of these analyses for non-cancer patients. Clinical EMR review included a specific focus on antibiotic use, exposure and timing of chemotherapy, and indices of disease severity (e.g. intensive care unit (36) admission, ventilator usage, death). The Fred Hutchinson Cancer Research Center (FHCRC) Institutional Review Board approved this study.

Definitions

Cases of VGS were considered proven when two or more blood cultures drawn at the same time were positive for a VGS; presumed cases were defined as patients with only a single positive blood culture.(7, 17, 24) Patients with multiple consecutive positive cultures for VGS were considered as a single episode of VGS when the same organism was identified within ≤ 4 weeks from the prior episode. Any patients with additional positive cultures beyond 4 weeks following the first event were considered an additional VGS event in these analyses. Antimicrobial sensitivities were performed using standardized E-test or Kirby-Bauer methods by the UWMC Microbiology Laboratory. Levofloxacin resistance was interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines.(37) Within the cancer population, data were evaluated separately for those with proven and all VSB events (Figure 1).

Cancer VGS patients who were undergoing their first cycle of chemotherapy and/or were recently diagnosed (≤30 days prior) were considered to have a new diagnoses; HCT patients were not considered to be new diagnoses due to the significant treatment history prior to transplantation. Fever was defined as reported body temperature ≥ 38.0°C and neutropenia as an ANC ≤ 500 cells/μL. Mucositis was categorized as mild, moderate or severe based upon mucositis scoring and/or characterization noted in the clinical record. Prophylactic antimicrobial use was defined as antibiotics that were administered for neutropenia. Empiric antimicrobials were defined as antibiotics that were administered for neutropenic fever or other signs of infection during neutropenia prior to the identification of the pathogen.

Renal failure or insufficiency was defined as an elevation of creatinine within 14 days of the VGS event greater than twice baseline and hepatic involvement was defined as the development of liver enzyme (ALT, AST) elevations greater than 4 times normal range. Septic shock was defined as hypotension (MAP $\leq$ 60mmHg) necessitating fluid resuscitation and treatment with vasopressors during the bacteremic event. Respiratory failure was defined as respiratory clinical signs necessitating intubation and mechanical ventilation. Death was considered attributable to the VGS event when death occurred $\leq$ 30 days after the date of VGS bacteremia and could be causally linked in the medical record to VGS. Death associated with VGS bacteremia was defined as death that occurred $\leq$ 30 days after detection but not directly associated with VGS.

Statistical analysis

We used the chi-squared test to compare the proportion of VGS cases with levofloxacin resistance in all groups, as well as demographic, clinical and outcome differences. When expected cell counts were < 5, Fisher's exact test was used. Mann-Whitney tests were used to compare any continuous variables between populations.

Univariate and multivariate logistic regression was performed to assess potential risk factors associated with levofloxacin resistance in the cancer VGS patients. Risk factors previously associated with fluoroquinolone resistance, including year (in 2 year intervals), receipt of HCT or chemotherapy associated with mucositis, primary oncological diagnosis, and fluoroquinolone prophylaxis were included *a priori* in the model. Additional explanatory risk factors such as age and sex were also included due to higher risk for older patients and prior gender-related associations to VGS infections. All *a priori* risk factors and additional exploratory risk factors were assessed in univariate analyses and included in the final model if they were found to be associated with fluoroquinolone resistance ($p<0.1$). Risk factors were entered simultaneously using standard regression analysis. SPSS Version 19 was used to perform all statistical analyses; 2-sided p value was considered statistically significant.

III. Results

We identified 261 patients with at least one positive blood culture for VGS between 1 January 2006 and 31 December 2012. Most (165/261 [63%]) patients had cancer; 69/165 (42%) were HCT recipients and 96/165 (58%) were oncology patients (Table 1). The median age of cancer patients was 53 years old (interquartile range [IQR], 42-62) and most had a primary diagnosis of a hematological malignancy (142/165 [86%]). Of the hematological malignancies, 92 of 165 (56%) patients had an acute leukemia, of which acute myeloid leukemia (AML) was the most frequent overall diagnosis in the cohort. Of the HCT population, the majority (48 of 69; 69.6%) had an allogeneic transplant. Of those receiving an allogeneic transplant, most (29/48 [60.4%]) had an unrelated donor transplant and most received peripheral blood stem cells (PBSC) as their stem cell source (54/69 [78.3%]).

Streptococcus viridans species

Of 165 VGS isolates, 70 were unclassified at the species level (42.4%). Of VGS that were identified at a species level, *S. mitis* was the most common species identified in the cancer population (72/95 [75.8%]). *S. milleri* (2/95 [2.1%]) was isolated only in the cancer population. A higher number of unclassified VGS cultures was identified in presumed as compared to proven cases (48/74 [65%] vs. 22/91 [24%], respectively; $p \leq 0.001$) while a higher number of *S. mitis* species were cultured in proven as compared to presumed cases (57/91 [63%] vs. 15/74 [20%], respectively; $p \leq 0.001$).

Antimicrobial resistance

Within the cancer population, levofloxacin resistance was higher in HCT recipients compared to other cancer patients (48/66 [73%] vs 51/99 [52%], respectively; p=0.033). Of cancer patients on levofloxacin prophylaxis, 96 of 106 (91%) had levofloxacin-resistant VGS compared to only 3 of 51 (6%) in those who did not receive levofloxacin prophylaxis (p=0.001). Antimicrobial sensitivities for the cancer population are shown in Figure 2. Antimicrobial sensitivities for the HCT and Hem/Onc populations indicated no differences for PCN, clindamycin, erythromycin, ceftriaxone and vancomycin resistance (data not shown). Levofloxacin resistance varied by taxonomic classifications of Viridans streptococci unclassified (40/97 [41%]), *S. mitis* (54/98 [55%]), *S. salivarius* (4/13 [30%]) and *S. parasanguinis* (3/10 [30%]).

Proven vs. presumed VGS events

HCT recipients had a higher number of presumed versus proven VGS events (39/74 [53%] vs. 30/91 [33%], respectively; p=0.011). No other differences were identified between proven and presumed VGS in the cancer patients in demographics, antimicrobial susceptibilities including levofloxacin resistance, clinical appearance, antibiotic use (including prophylactic fluoroquinolone use) and severity of VGS events, therefore, both proven and presumed VGS were combined for the remainder of the analyses.

Clinical presentation

The majority of patients with VGS had a fever 133/165 (80.6%) and were neutropenic 121/165 (73.3%), however the Hem/Onc population had a higher incidence of fever 84/96 (87.5%) than the HCT population 49/69 (71.0%; p=0.010) (Table 2). HCT and Hem/Onc populations were similar in days from chemotherapy/induction to fever at 12.5 (IQR 10, 16) and 13 (IQR 11, 17) days, respectively. Mucositis was reported in 36.4% (60/165) of cancer patients, with a higher proportion in HCT patients 45/69 (65.2%) than Hem/Onc patients 15/96 (15.6%; p<0.001).

Antibiotic use

Prophylactic antibiotics were administered in 134 of 165 (81%) cancer patients and levofloxacin prophylaxis was the most common in 110 of 165 (67%) patients. Levofloxacin prophylaxis was frequently administered in both HCT and Hem/Onc populations, though was more common in HCT patients (53/69 [76.8%] vs. 57/96 [59.3%]; p=0.019). Empiric antibiotics were administered to 124 of 165 (75.2%) cancer patients with VGS, and in similar distributions of HCT and Hem/Onc patients (50/69 [72.5%] and 74/96 [77.1%], respectively; p=0.5). The most frequent empiric therapies were ceftazidime and ceftazidime/vancomycin among the entire group with similar rates of ceftazidime use between HCT and Hem/Onc populations and increased empiric vancomycin use in the Hem/Onc as compared to the HCT group (p=0.019). Vancomycin was the most frequently administered therapy for documented VGS in cancer patients (155/165 [93.9%]).

Severity of VGS

We evaluated the correlates of disease severity in cancer patients with VGS. Most patients were hospitalized a median length of stay of 10 days (IQR 5, 23.5), but durations of stay were significantly longer in HCT than Hem/Onc patients (19 days versus 8 days for; p=0.001) (Table 2). Of the 39 of 165 (23.4%) cancer patients admitted to the Intensive Care Unit (ICU), rates between HCT (17/69 [25%]) and Hem/Onc (22/96 [23%]) populations with VGS were similar (p=0.85). Respiratory failure, septic shock, renal failure, need for hemodialysis and acute liver injury developed in a similar number in both HCT and Hem/Onc populations (Table 2). A total of 80 of 165 (48.5%) of patients in the cancer population with VGS died during follow-up with a total of 14 of 80 (17.5%) attributable and associated with the VGS event; 6 of 80 (3.2%) were directly attributable and 8 of 80 (4.8%) were associated with VGS bacteremia. There was no difference in mortality between HCT (31/69 [45%]) and Hem/Onc (49/96 [51%]) populations. Overall mortality was higher in the VGS patients with levofloxacin resistance as compared to those without resistance (47.1% vs. 27.9%, p=0.018); those with levofloxacin resistance had a two-fold higher risk of death attributable and associated to VGS as compared to those without levofloxacin resistant VGS (12.0% with resistance vs. 6.7% without resistance; p=0.362). Overall mortality and death associated/attributable occurred in both proven and presumed cases at similar rates (overall mortality: 52.5% vs 47.5%, p=0.55; associated/attributable: 64.3% vs 35.7%, p=0.33).

Risk factors for levofloxacin resistant VGS in Cancer and HCT patients

Calendar year was significantly associated with levofloxacin resistance in univariate analyses, as VGS events occurring in 2011 and 2012 had increased odds of being levofloxacin resistant VGS (OR 5.8 [95% CI 2.17 to 15.5]) when compared to 2006

(Table 3). Patients with VGS who had received chemotherapy associated with the development of mucositis, an acute leukemia (AML/ALL) or HCT had significantly increased odds for levofloxacin resistance (Table 3). Age or gender was not associated with levofloxacin resistance. Patients with VGS who had received prophylactic levofloxacin had the highest odds of any sub-group for having levofloxacin resistant VGS (OR 154 [95% CI 40.4 to 584]) (Table 3).

In the multivariate model evaluating risk factors for levofloxacin resistance in patients with VGS, receipt of levofloxacin prophylaxis was the only risk factor associated with increased odds for the detection of levofloxacin resistant VGS (OR 398 [95% CI 43.6 to 3628]) (Table 3). In order to better understand other associated risk factors in those with a high rate of levofloxacin use, we repeated our multivariate model excluding prophylactic levofloxacin (Table 3). In this second model, VGS occurring in 2009 and 2010 (OR 4.2 [95% CI 1.02 to 17.7]) and in 2011 and 2012 (OR 7.1 [95% CI 2.1 to 23.7]) had increased odds of levofloxacin resistant VGS when controlling for all other variables. Neither mucositis-inducing chemotherapy nor HCT were associated with the development of levofloxacin resistant VGS in either multivariate model.

VGS in cancer vs. non-cancer populations

Frequency of cases of VGS increased across the years of observation overall, predominantly reflecting the increase in the cancer population (Figure 3). The percentage of patients with VGS that were cancer patients increased from 2006 to 2012 (Figure 3). In 2006, 25/39 (64%) of VGS were cancer patients, increasing to 33 of 48 (69%) in 2011 and 43 of 54 (80%) in 2012 (Figure 3).

Streptococcus viridans species

The most common VGS reported in both cancer and non-cancer patients were viridans streptococci unclassified (43/99 [43.4%] and 70/165 [42.4%], respectively); similar rates were found in both populations (p=0.90) (Figure 4). Of VGS that were identified at a species level, *S. mitis* was significantly higher in the cancer population when compared to the non-cancer population (72/95 [75.8%] vs 29/55 [52.7%], respectively; p=0.004). *S. sanguinis* (4/55 [7.3%]) was cultured only in the non-cancer population.

Antimicrobial resistance

Antimicrobial sensitivities for VGS in the cancer and non-cancer populations indicated no differences for penicillin (PCN), clindamycin, erythromycin, ceftriaxone and vancomycin (Figure 2). However, we found a significant difference in levofloxacin resistance between the cancer and non-cancer populations (99/157 [63%] vs 2/85 [2%], respectively; $p \leq 0.001$) (Figure 5).

IV. Discussion

Our study identified increasing numbers of VGS detected from blood cultures from 2006-2012 at our large tertiary care center. We demonstrated that the increase in numbers was exclusively noted in cancer and HCT recipients. In addition, resistance to levofloxacin was seen almost exclusively in these patients, particularly those with underlying hematologic malignancies and those undergoing HCT, and appears to be driven by the use of levofloxacin prophylaxis for neutropenia.

Prophylactic antibiotics for cancer patients with neutropenia are currently recommended within cancer care guidelines, as they have been shown to decrease morbidity and mortality associated with GNR infections.(1, 8-10) Many studies have identified that the use of prophylactic antibiotics, particularly fluoroquinolones, result in decreased infection rates, decreased incidence of fever, decreased hospitalization and decreased costs, some without the emergence of resistance.(6, 32, 38)

As risk factors for VGS in cancer patients have been well described (2, 3, 7, 21, 22), we focused instead on the changing frequency of VGS over time and on the emergence of fluoroquinolone resistance. We were able to document an increasing number of VGS events and fluoroquinolone resistance among patients within our tertiary medical center that was driven by cancer and HCT patients. Interestingly, the general population at UWMC includes a large number of patient populations that either received extensive antibiotic exposure (e.g. cystic fibrosis, endocarditis, ICU patients) or are other high-risk immunocompromised patient populations (e.g. solid organ transplant recipients) yet rates of resistance remained low.

Levofloxacin resistance was almost exclusively seen in cancer and transplant patients, and resistance in the general medical population at this tertiary center was rare. When these data were evaluated by calendar year, the percentage of levofloxacin resistant VGS increased in the cancer populations throughout the study period. By comparison, a study from our center evaluated the transition from ceftazidime to levofloxacin neutropenic prophylaxis in the HCT population.(32) In this study, during the first years post-transition, rates of VGS remained low. Since the large prospective randomized placebo-controlled trial that demonstrated benefits of levofloxacin prophylaxis was first published in 2005 (38), there have been few studies that have evaluated the emergence of resistance patterns with application of standardized use of such prophylaxis. Looking at these more recent data, one can hypothesize that with publication of this trial, use of prophylaxis expanded through the local cancer community, leading to significant fluoroquinolone exposures in these patients that would increase the risk for resistance. Consistent with this hypothesis, hematologic malignancy patients and HCT recipients were the most likely to develop neutropenia during therapy, to receive levofloxacin and were at increased risk for VGS. The small number of patients within the cohort who developed VGS during the first ever cycles of chemotherapy had no evidence of fluoroquinolone resistance.

By comparison, a number of other studies have evaluated antimicrobial resistance in VGS in larger hospital settings and in other high-risk populations. In 2006, Yap reported that VGS found in patients without cancer were all susceptible to levofloxacin though with varying levels of susceptibility to other antimicrobial agents.(40) Others have noted increasing rates of resistance to PCN and erythromycin.(41, 42) VGS detected in sputum of cystic fibrosis patients were found to have high levels of resistance to PCN and erythromycin, but fluoroquinolone resistance was either not reported or not found.(43, 44) Interestingly, some of these same studies do report higher proportions of patients with reduced susceptibility but not outright resistance to fluoroquinolones.(41, 43)

Previous studies in cancer populations have indicated increased fluoroquinolone resistance with the use of these agents as prophylaxis.(27, 34, 45, 46) Mechanistically, prophylaxis for neutropenia is known to increase minimum inhibitory concentrations (MIC) to levofloxacin in numerous gastrointestinal (GI) gram-negative and gram-positive organisms.(45) VGS in the oropharynx of HCT patients during fluoroquinolone prophylaxis are also associated with significant reductions in fluoroquinolone susceptibility when compared to pre-exposure samples.(34) Such antimicrobial resistance changes in the oral and GI microbiome provide a link between pathologic changes in the mucosa seen during cancer therapy, antibiotic exposure, bacterial invasion and the development of breakthrough VGS.

The multivariate analysis supports that neutropenic prophylaxis is a major influence on the development of fluoroquinolone resistance in cancer patients with VGS. Levofloxacin prophylaxis was the only significant predisposing factor linked to the detection of fluoroquinolone-resistant VGS. As levofloxacin prophylaxis was the driving force behind the detection of resistant VGS, it was important to evaluate the trends in both levofloxacin prophylaxis and resistance over the study period. Evidence of levofloxacin prophylaxis in patients with documented VGS also increased during the study period with 44% on prophylaxis in 2006, 80% in 2010, 88% in 2011 and 71% in 2012. In these same patients with VGS, levofloxacin resistance significantly increased from the beginning of the study period, 2006 to 2012. Evaluation by linear regression found a difference in levofloxacin resistance by year ($p<0.001$) but did not find a difference in levofloxacin prophylaxis by year ($p=0.38$).

In order to address what other factors might be associated with levofloxacin resistance in VGS patients, we removed levofloxacin prophylaxis from our model. Not surprisingly, patients with hematological malignancies were more likely to receive levofloxacin prophylaxis (75% v. 13%) and have levofloxacin resistance (72% v. 5%) when compared to those with solid tumors. When evaluating oncologic diagnoses more specifically, risk was dominated by patients with acute leukemias (AML and ALL). Interestingly, in these multivariate analyses, HCT was not a risk factor for development of levofloxacin-resistant VGS. This lack of association is somewhat surprising, as most HCT recipients have had significant prior antibiotic exposure and are likely to develop mucositis. The increase in non-myeloablative transplantation, more aggressive regimens for mucosal protection, and differences in antibiotic exposure of HCT recipients are possible reasons for these differences.

There were limitations to our study as are common in such high-risk populations. Though we did identify an increase in the frequency of VGS infections within the cancer population at the center, we could not determine the incidence of VGS. The wide variety of patients seen in an outpatient clinic, challenges in identifying periods of risk and the variations in outpatient treatment precluded our ability to identify a true denominator of patients potentially at risk; similar issues exist within the general medical population in this study. Identifying incidence trends for VGS over this time period would provide stronger support for the emergence of VGS infections within the cancer population. While it is possible that increases seen are related to a growing cancer population seen at the center, the near doubling of cases (Figure 2) suggests that is unlikely the case.

Perhaps the most significant weakness in our study was the inability to account for the overall antibiotic exposure of patients prior to their development of VGS. While we were able to capture antibiotic use immediately prior and following bacteremia, significant exposures likely occurred for most patients during multiple prior treatment courses and may have a major effect on the incidence of resistance. Future studies addressing total antibiotic exposure will be important to understanding the development of fluoroquinolone-resistant VGS in these populations.

In summary, our study demonstrated increasing number of episodes of VGS bacteremia and VGS-associated fluoroquinolone resistance at our large tertiary medical center driven by fluoroquinolone prophylaxis in cancer and HCT patients. The expansion of fluoroquinolone-resistant VGS seen in our study should serve as warning to providers managing cancer patients that increased awareness is needed when treating patients who develop gram positive bacteremia while on levofloxacin prophylaxis. A re-evaluation of the current use of fluoroquinolones as neutropenic prophylaxis in cancer and HCT patients may be necessary if current trends continue.

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Appendix A: Tables

Table 1: Demographics of Cancer, HCT and Hem/Onc patients with VGS bacteremia from 2006-2012

	All Cancer n=165	HCT n=69	Hem/Onc n=96	p-value*
Age (yrs), median (IQR)	53 (42, 62)	51 (37, 61)	55 (44, 64)	0.103
Gender, n (%)				
Male	97 (58.8)	41 (59.4)	56 (58.3)	0.889
Female	68 (41.2)	28 (40.6)	40 (41.7)	
Race, n (%)				
White	143 (86.7)	59 (85.5)	84 (87.5)	
Black	6 (3.6)	3 (4.3)	3 (3.1)	0.445
Asian	6 (3.6)	3 (4.3)	3 (3.1)	
Native American	4 (2.4)	3 (4.3)	1 (1.0)	
Pacific Islanders	6 (3.6)	1 (1.4)	5 (5.2)	
Ethnicity, n (%)				
Yes Hispanic	8 (4.8)	2 (2.9)	6 (6.3)	0.470
No	157 (95.2)	67 (97.1)	90 (93.8)	
Primary Onc Dx, n (%)**				
AML	71 (43.0)	20 (29.0)	51 (53.1)†	
ALL	21 (12.7)	11 (15.9)	10 (10.4)	
CML	5 (3.0)	5 (7.2)	0 (0)†	
CLL	1 (0.6)	0 (0)	1 (1.0)	<0.001***
MM	13 (7.9)	8 (11.6)	5 (5.2)	
NHL	16 (9.7)	16 (23.2)	0 (0)†	
HL	2 (1.2)	2 (2.9)	0 (0)	
MPLD	10 (6.1)	6 (8.7)	4 (4.2)	
Other	3 (1.8)	1 (1.4)	2 (2.1)	
Solid tumor	23 (13.9)	0 (0)	23 (24.0)†	
Category Onc Dx, n (%)				
Heme/Onc	142 (86.1)	-	-	
Solid tumor	23 (13.9)	-	-	
New Diagnosis, n (%)				
Yes	15 (9.1)	0 (0)	15 (15.6)	
No	150 (90.9)	69 (100)	81 (84.4)	

Abbreviations: VGS, Viridans group streptococci; HCT, hematopoietic cell transplant; Hem/Onc, hematology/oncology; IQR, interquartile range; Onc, oncology; Dx, diagnosis
*Associations between HCT and Hem/Onc patient populations
**AML=acute myeloid leukemia, ALL=acute lymphoid leukemia,
CML=chronic myeloid leukemia, CLL=chronic lymphoid leukemia,
MM=multiple myeloma, NHL=non-Hodgkin's lymphoma,
HL=Hodgkin's lymphoma, MPLD=myeloproliferative disorders
***Statistically significant at p-value ≤0.05 with Chi-squared test (R x C contingency table)
†Adjusted residuals > 2.0 and -2.0, identifying greatest differences between these diagnoses

Table 2: Clinical, therapeutic and outcome data for cancer patients with VGS

	Total n=165	HCT n=69	Hem/Onc n=96	p value
Fever, n (%)	133 (80.6)	49 (71.0)	84 (87.5)	0.01*
Mucositis, n (%)	60 (36.4)	45 (65.2)	15 (15.6)	<0.001**
Neutropenic, n (%)	121 (73.3)	54 (78.3)	67 (69.8)	0.23
Levo Prophy, n (%)	110 (66.7)	53 (76.8)	57 (59.4)	0.019*
GI meds, n (%)	115 (69.7)	54 (78.3)	61 (63.5)	
PPI	111 (67.3)	54 (78.3)	57 (59.4)	0.029**
H2 blocker	4 (2.4)	0 (0)	4 (4.2)	0.12
TPN, n (%)	51 (30.9)	42 (60.9)	9 (9.4)	<0.001**
LOS in days, median (IQR), range	10 (5, 23.5) 0-137	19 (8.5, 30)	8 (5, 15)	<0.001***
ICU, n (%)	39 (23.6)	17 (24.6)	22 (22.9)	0.8
ICU days, median (IQR)	6 (2, 10.5)	7.5 (4.5,18.5)	3 (2, 9.5)	0.07
Septic shock, n (%)	23 (13.9)	8 (11.6)	15 (15.6)	0.46
Death, n (%)	80 (48.5)	31 (44.9)	49 (51.0)	0.4
Attributable/Associated	14 (8.5)	5 (7.2)	9 (9.4)	0.8

Abbreviations: VGS, Viridans group streptococci; HCT, hematopoietic cell transplant; Levo, levofloxacin; TPN, total parenteral nutrition; LOS, length of hospital stay; ICU, intensive care unit; IQR, interquartile range
* Statistically significant between HCT and non-HCT 2-sided Fisher's exact test
**Statistically significant between HCT and non-HCT 2-sided Pearson Chi-Square test
***Statistically significant between HCT and non-HCT 2-tailed Mann-Whitney test

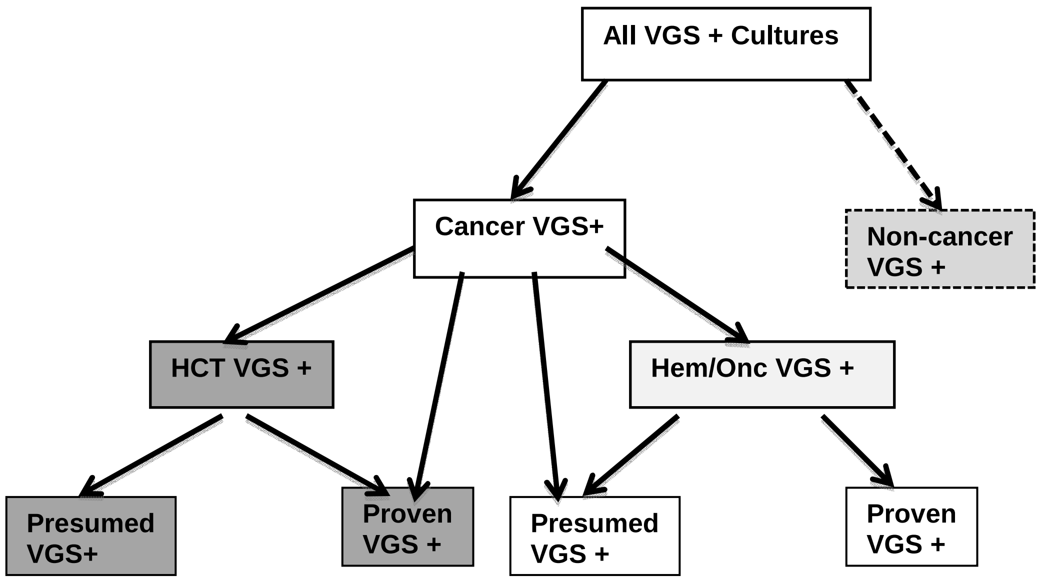
Table 3: Univariate and multivariate analysis of risk factors for levofloxacin-resistant VGS in cancer patients (n=157)

	Levofloxacin Resistance			Multivariate	
	Susceptible n=58 n/total (%)	Resistant n=99 n/total (%)	Univariate	With Levo Prophylaxis OR (95% CI)	Without Levo Prophylaxis OR (95% CI)
Age					
≥60	21/58 (36)	28/99 (28)	ref	---	---
40-59	24/58 (41)	46/99 (46)	1.44 (0.68-3.05)	---	---
<40	13/58 (22)	25/99 (25)	1.44 (0.60-3.47)	---	---
Gender (female)	20/58 (34)	45/99 (45)	1.58 (0.81-3.10)	---	---
Year					
2006	15/58 (26)	10/99 (10)	ref	ref	ref
2007 and 2008	13/58 (22)	11/99 (11)	1.27 (0.41-3.94)	0.22 (0.02 -2.42)	1.86 (0.44 -7.82)
2009 and 2010	15/58 (26)	20/99 (20)	2.00 (0.71-5.68)	0.81 (0.07-9.94)	4.24 (1.02-17.7)*
2011 and 2012	15/58 (26)	58/99 (59)	5.80 (2.17-15.5)	4.12 (0.51-33.1)	7.12 (2.14-23.7)*
HCT	18/58 (31)	48/99 (48)	2.09 (1.06-4.14)*	0.90 (0.17-4.65)	2.20 (0.75-6.45)
Chemo w/ mucositis	30/41 (73)	87/99 (88)	2.66 (1.06-6.65)*	0.61 (0.08-4.76)	2.74 (0.86-8.69)
Primary Dx					
Others**	18/58 (31)	28/99 (28)	ref	ref	ref
Acute Leukemia	20/58 (34)	70/99 (71)	2.25 (1.04-4.87)*	5.42 (0.85-34.7)	1.97 (0.66-5.88)
Solid tumor	20/58 (34)	1/99 (1)	0.03 (0.004-0.26)*	0.67 (0.03-15.4)	0.11 (0.01-1.2)
Levo Prophylaxis	10/58 (17)	96/99 (97)	154 (40.4-584)*	398 (43.6-3628)*	---

Abbreviations: VGS, Viridans group streptococci; OR, odds ratio; CI, confidence interval; ref, reference; HCT, hematopoietic cell transplant; Dx, diagnosis; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; Levo, levofloxacin; Reference groups: Gender, male=0; HCT, Non-HCT recipients or Hem/Onc=0; Chemo w/ mucositis, No=0; Levo prophy, No=0 *Statistically significant OR with 95% CI not including 1; **Others includes CML, CLL, MM, NHL, HL, MPLD, and other cancers

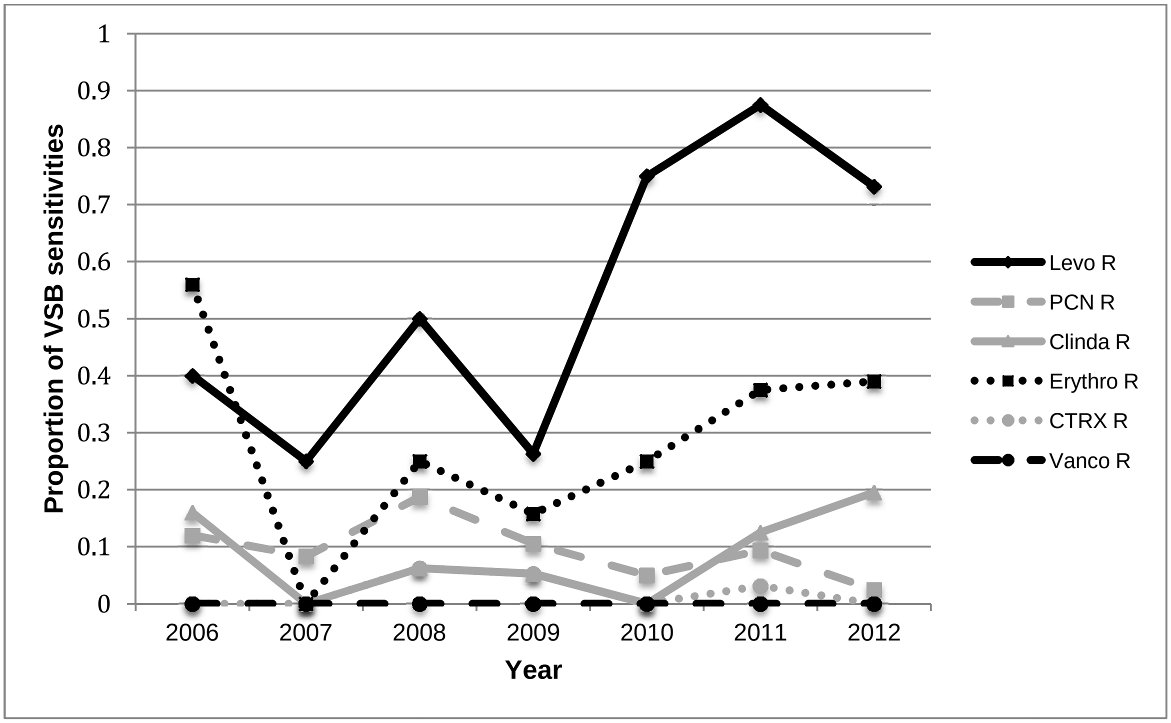
Appendix B: Figures

Figure 1: Flow diagram of categorization of patients with Viridans group streptococcal bacteremia



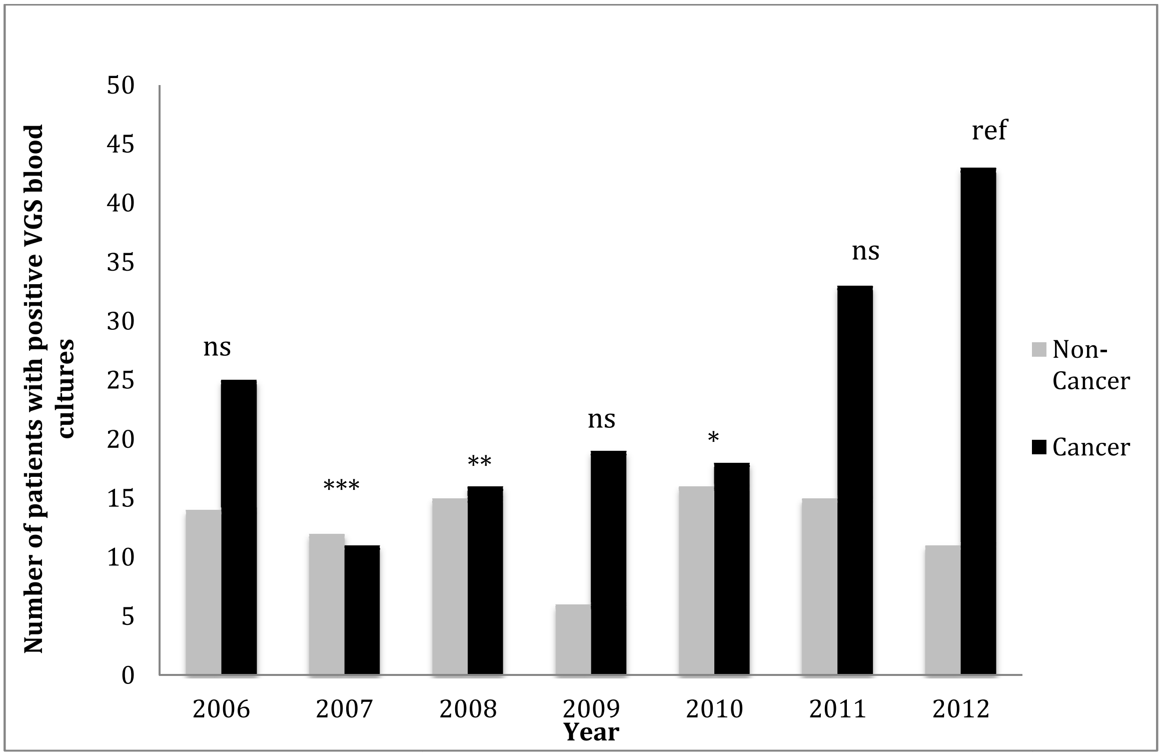
Abbreviations: VGS, Viridans group streptococci; Hem/Onc, Hematology/Oncology patients; HCT, Hematopoietic cell transplant recipients

Figure 2: Antimicrobial resistance by year of VGS event in the cancer population



Abbreviations: VGS, Viridans group streptococci; Levo, levofloxacin; PCN, penicillin; Clinda, clindamycin; Erythro, erythromycin; CTRX, ceftriaxone; Vanco, vancomycin

Figure 3: Number of VGS events in cancer and non-cancer populations from 2006 to 2012



Abbreviations: VGS, Viridans group streptococci. Fisher's exact test comparing the Cancer VGS by calendar year 2012, ns=non-significant; ref=reference; *=0.01; **=0.008; ***=0.007

Figure 4: Taxonomic distribution of VGS in the cancer and non-cancer populations from 2006 to 2012

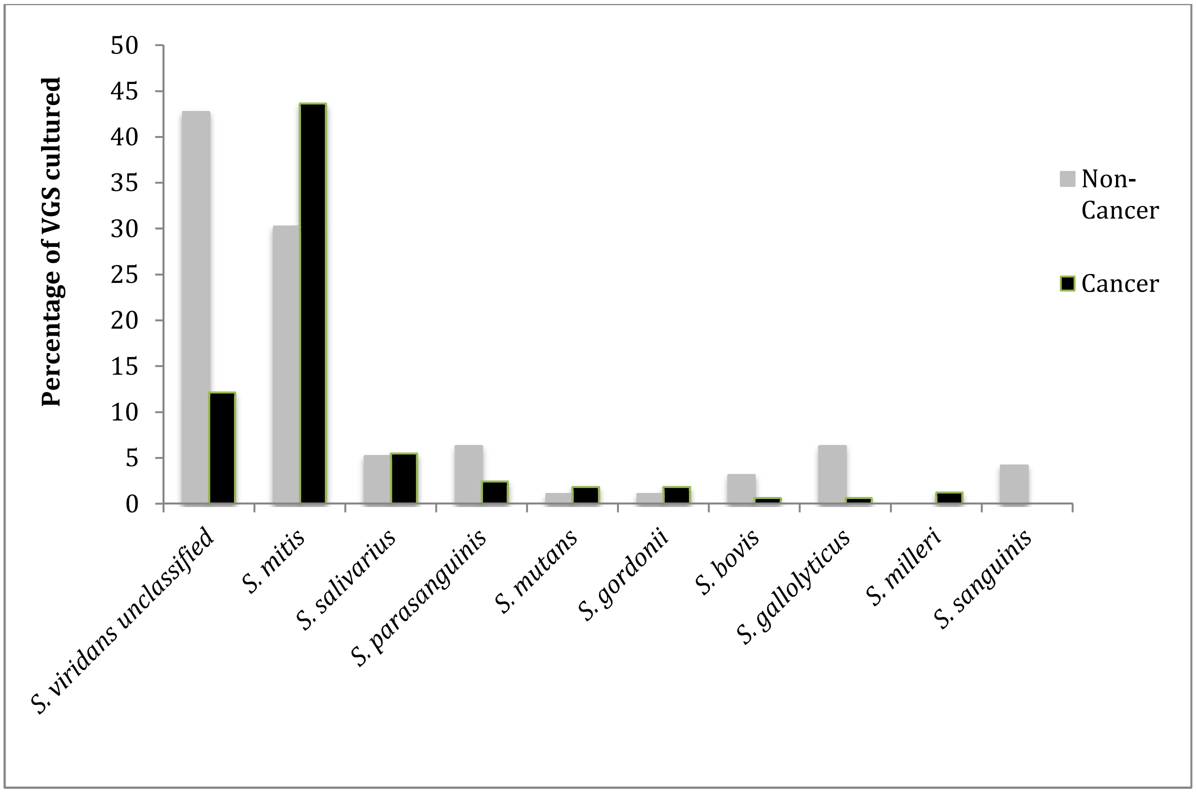
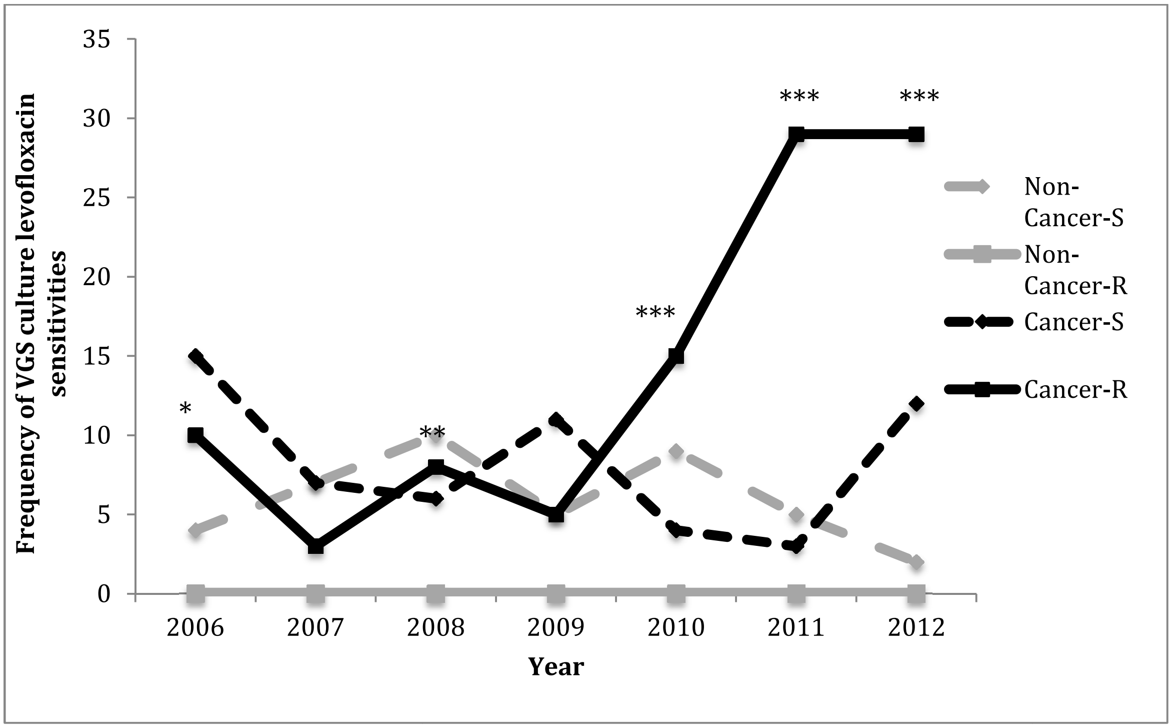


Figure 5: Levofloxacin susceptibilities by calendar year of VGS events for cancer and non-cancer populations



Abbreviations: VGS, Viridans group streptococci; S, sensitive; R, resistant
Statistically significant difference between Cancer-R and non-Cancer-R to levofloxacin * p=0.015, **p=0.001, ***p≤0.001